Supplementary Files

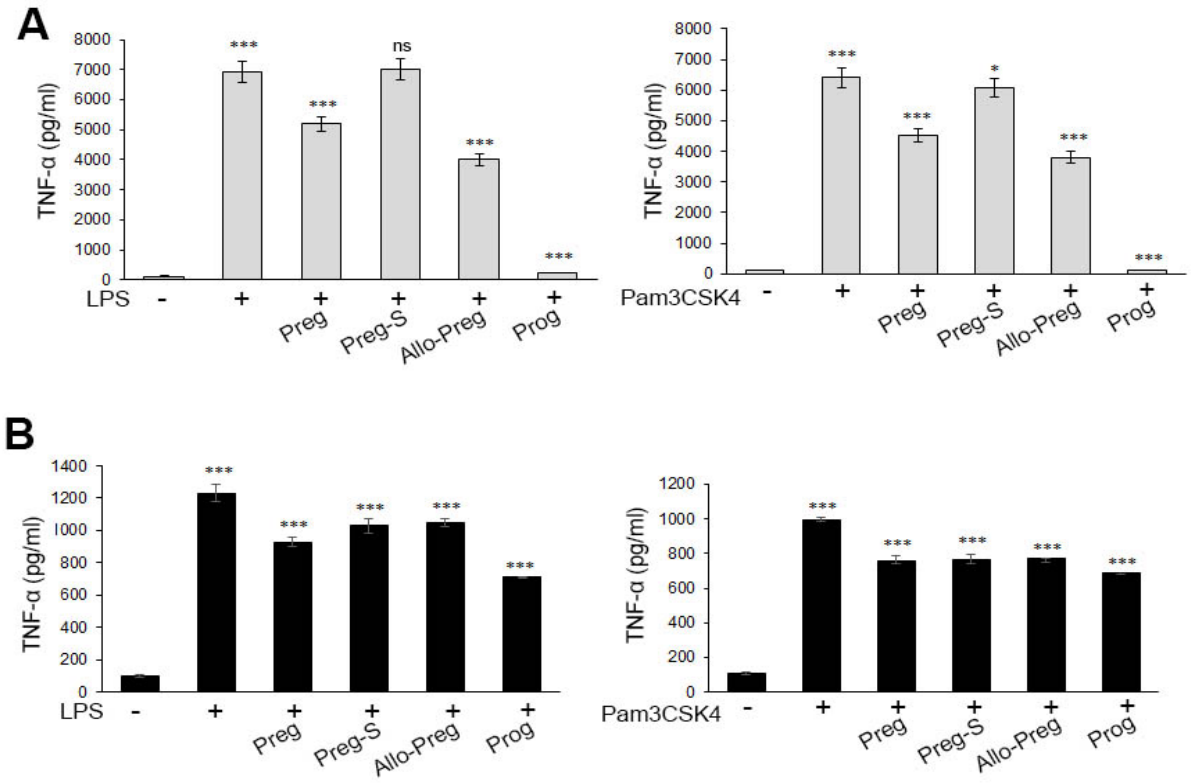
Supplementary Table 1

Ratio of F527 (YFP)/F475 (CFP)

	Cell alone	**Cell + DMSO**	**Cell + P5**
CFP YFP	1.3078 ± 0.266	1.4965±0.1770	1.3367±0.221
CFP CP YFP	0.65225± 0.253	0.636625±0.24470	0.48123±0.1213
CFP CP K1 YFP	0.393625±0.0808	0.414385±0.079	0.415115±0.089

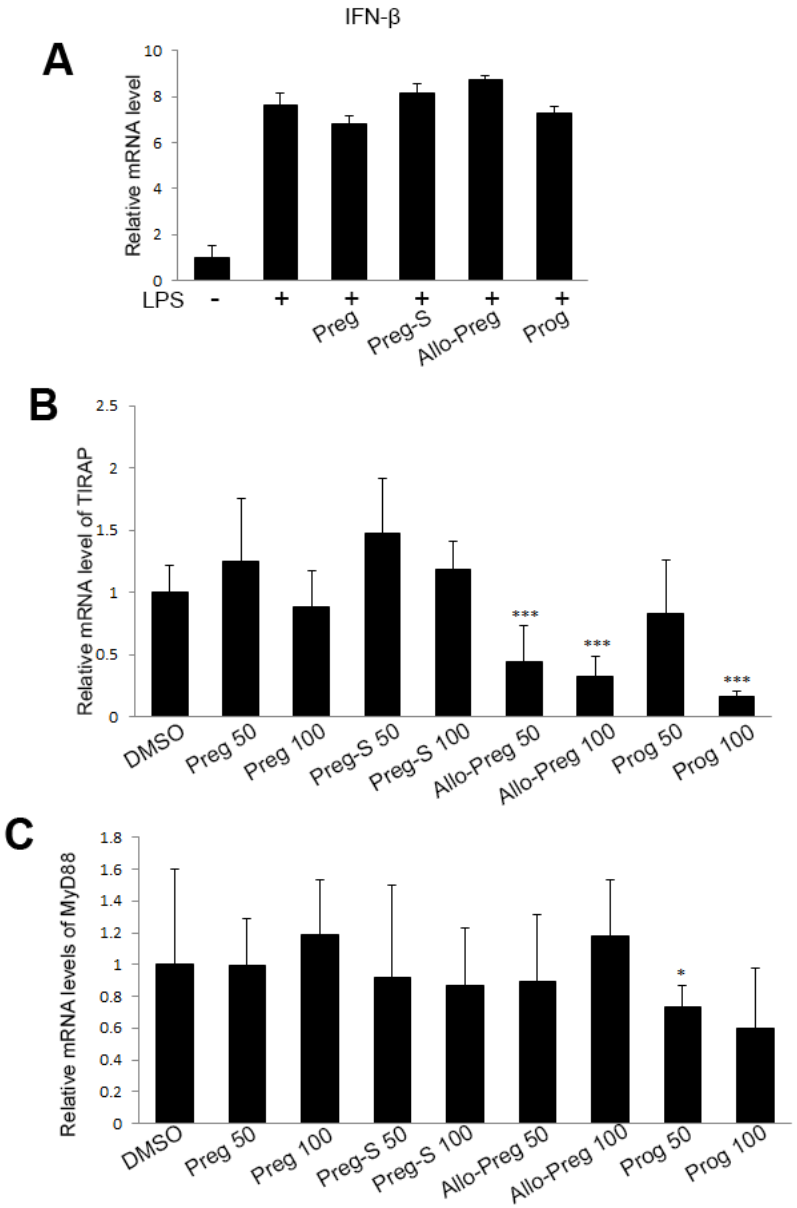
Supplementary Table 1. FRET analysis to examine the activation of CLIP170 by pregnenolone. HEK293T cells were transfected with eukaryotic expression plasmids harbouring CFP-YFP or YFP-CLIP-170-CFP or YFP-CLIP-170-K1-CFP fusions. Twenty-four hours after the transfections, cells were lysed and the lysates were incubated with pregnenolone or DMSO followed by spectral analysis with excitation at 425 nm. CFP acted as donor and YFP served as the acceptor. The cell lysate that expressed CFP-YFP tandem exhibited marked YFP (acceptor) emission after the CFP (donor) excitation due to FRET. The cell extract that contains YFP-CLIP170-CFP fusion exhibited higher ratio of fluorescence at YFP emission as compared to YFP-CLIP-170-K1-CFP. The ratio of YFP to CFP fluorescence emission was decreased upon treatment of YFP-CLIP170-CFP lysate with pregnenolone in comparison with DMSO vehicle control. Mean ±s.d. was determined from three independent sample measurements.

Supplementary Figure 1



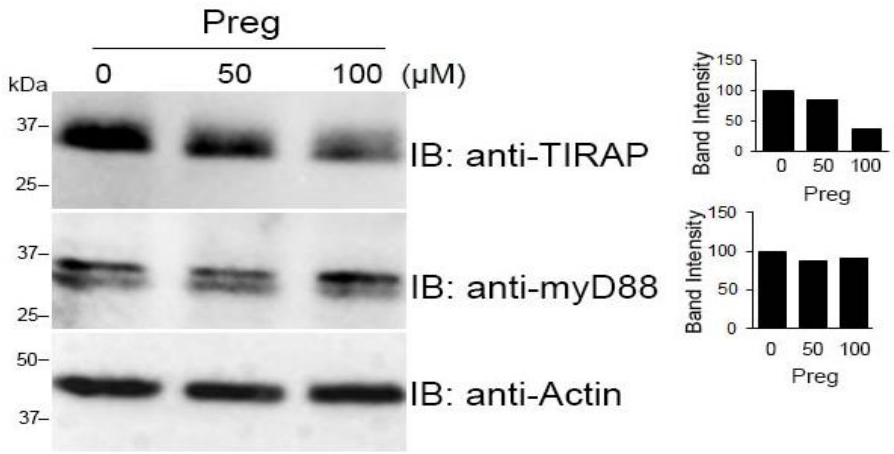
Supplementary Figure 1. Pregnenolone or its metabolic derivatives supresses LPS or Pam3CSK4-induced TNF-α secretion in BMDM (*A*) and N9 microglial cells (*B*). BMDM or N9 microglial cells were treated with pregnenolone or its metabolites (50 μM) followed by stimulation with LPS or Pam3CSK4. Next, the levels of secreted TNF-α was quantified by ELISA. Pregnenolone or its metabolites supressed the LSP or Pam3CSK4-induced TNF-α secretion in BMDM and in N9 microglial cells. Data are presented as mean ±s.d. from at least three independent experiments (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$); ns: not significant.

Supplementary Figure 2



Supplementary Figure 2. (*A*) Pregnenolone or its metabolic derivatives did not supresses LPS-induced IFN-β in RAW264.7 cells. RAW264.7 cells were treated with pregnenolone or its metabolites (50 μM) followed by stimulation with LPS. Next, the levels of IFN-β was quantified by qPCR. **(*B&C*) Effect of Pregnenolone or its metabolites on expression of *TIRAP* and *MyD88* in RAW264.7 cells**. RAW264.7 cells were treated with pregnenolone or its metabolites (50 μM) followed by quantification of *TIRAP* (B) or *MyD88* (C) level by qPCR. Allopregnenolone or progesterone suppressed the expression of *TIRAP*. Data are presented as mean ±s.d. from at least three independent experiments (**P* < 0.05 and ****P* < 0.001).

Supplementary Figure 3



Supplementary Figure 3. Pregnenolone induces degradation of TIRAP in peritoneal macrophages. Immunoblotting of mouse peritoneal macrophages treated with pregnenolone. Pregnenolone induced the degradation of endogenous TIRAP, whereas it did not affect the level of MyD88. Immunoblots are representative of two independent experiments. The right panels of the immunoblots show the densitometry analysis of the represented immunoblots where the test protein bands normalized to actin.